Anti-Chlamydial Antibodies In Women with Ectopic Pregnancy

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ABSTRACT

Background: Chlamydia trachomatis infection is the most common sexually transmitted bacterial infection worldwide. It can lead not only to significant morbidity, but it can also affect a woman's fertility. Patients with ectopic pregnancy are more likely to have immunoglobulin G (IgG) antibodies against Chlamydia trachomatis when compared with pregnant controls.

Aim of study: To compare the frequency of chlamydia trachomatis infection in women with ectopic and with normal pregnancy.

Methods: A case control study that was conducted in the Department of Obstetrics and Gynecology at Azadi Teaching Hospital / Kirkuk-Iraq during the period of 10 months from 1st of Feb till 1st of Dec 2019. It included 86 pregnant women selected from outpatient clinic and those who were admitted to the emergency department. They were divided into two groups: Case group included 43 women diagnosed with ectopic pregnancy and control group included 43 women with early normal intra uterine pregnancies (1^{st} trimester). Women using an intrauterine contraceptive device at the time of conception or had previous history of ectopic pregnancy, infertility, tubal surgery or smoking were excluded . After obtaining verbal consent from them five ml of venous blood was collected from all women to investigate for serum anti chlamydial IgG antibody titer.

Results: In this study, no statistical significant differences were found between study groups regarding age, gestational age, body mass index, and parity. We noticed that 17 (39.5%) of patients who diagnosed with ectopic pregnancy showed positive results of anti-chlamydial

antibody; while 7 (16.3%) of controls showed positive results of anti-chlamydial antibody. while 7 (16.3%) of controls showed positive results of anti-chlamydial antibody and the mean of anti-chlamydial antibody titer was significantly higher in patients who diagnosed with ectopic pregnancy than that in controls. Anti-chlamydial antibody titer > 9.98 NTU can help us predicting the risk of an ectopic pregnancy. No statistical significant correlations between anti-chlamydial antibody titer and all of age, gestational age, and body mass index.

Conclusion: Higher proportion of women with ectopic pregnancy had positive anti-chlamydial antibody than those with normal pregnancy. Anti-chlamydial antibody titer may play an important role to predict the risk of ectopic pregnancy as the mean of anti-chlamydial antibody titer was significantly higher in patients who diagnosed with ectopic pregnancy than that in women with normal pregnancy regardless age, gestational age or parity.

INTRODUCTION

An ectopic or extra uterine pregnancy is one in which the blastocyst implants anywhere other than the endometrial lining of the uterine cavity. Nearly 95 percent of EP implant in the fallopian tube. Bilateral EP are rare, and their estimated prevalence is one of every 200,000 pregnancies [(1)].a maternal mortality of 0.2 /1000 estimated Eps in United kingdom [(2)]

Over 95% of EPs are tubal in origin, with 80% located in the ampullary portion of the fallopian tube [(3)].

Only about 50% of women diagnosed with an EP have identifiable risk factors include [(4)]:Previous history of EP, History of pelvic surgery, Pelvic inflammatory disease [PID] and Chlamydia trachomatis, Smoking, Intrauterine contraceptive devices, Diethylstilbestrol exposure in utero, Assisted reproductive technology, Maternal age. The C. trachomatis infection is the most common sexually transmitted bacterial infection worldwide [(5)]. The chlamydial infection produces less severe symptoms than other sexua lly transmitted diseases [(6)]). These mild symptoms allow the infection to go unnoticed, ' with minimal patient awareness, until the secondary or the tertiary symptoms develop.

Serious sequelae like acute salpingitis and pelvic inflammatory disease often occur in association with repeated or persistent infections [7].

Despite widespread screening and treatment programs, the Chlamydia epidemic continues unabated with yearly increases in the number of reported cases . Urogenital chlamydial infection can lead to serious adverse outcomes in women, e.g. PID which can result in tubal factor infertility, ectopic pregnancy and chronic pelvic pain. Urogenital chlamydial infections do not result in any sustained immunity [8] The infection which is caused by C. trachomatis results in the formation of antibodies which are detectable in the serum in infected patients. Chlamydia antibodies are antibodies targeting bacteria of the genus Chlamydia, but it refers specifically to antibodies targeting Chlamydia trachomatis,

which is the cause of chlamydia infection in humans [9].

For lower genital tract infection, the detection of specific antibodies in a single serum specimen is held to be of little value because such antibodies are frequently found in sera from women who do not have active infection. Despite the difficulty of differentiating between previous and current lower genital tract infection, there is a considerable amount of evidence that the presence of these antibody is significantly associated with upper genital tract infection, particularly when the antibody is at a high titer [10].

Acute inflammation has been implicated in the tubal damage that predisposes to EP. Chronic salpingitis and salpingitis isthmica nodosa also contribute. Recurrent chlamydial infection causes intraluminal inflammation, subsequent fibrin deposition, and tubal scarring. Moreover, persistent chlamydial antigens can trigger a delayed hypersensitivity reaction that promotes continued scarring despite negative culture results. Inflammation within the fallopian tube can also arrest embryo progress and provide a premature pro-implantation signal [11].As the fallopian tube lacks a submucosal layer beneath its epithelium.

Therefore, a fertilized ovum can easily burrow through the epithelium and implant within tube's muscularis layer. As rapidly proliferating trophoblasts erode the muscularis layer, maternal blood pours into the spaces within the trophoblastic or the adjacent tissue [12]

A variety of studies found a higher proportion of women with EP will have serological evidence of previous infection with Chlamydia trachomatis as compared to pregnant without previous EP.

In addition, ectopic pregnant women who were seropositive for exposure to Chlamydia trachomatis had an increased prevalence of pelvic adhesions compared with ectopic patients who were negative for Chlamydia antibodies. There was also, as has been previously reported, a significant association between the severities of tubal damage with regards to pelvic adhesions and increasing antibody titer. This implies that increasing antibody titers are quantitatively related to both the presence of tubal damage and the severity of tubal damage [13].

PATIENTS AND METHODS

This is a case control study that was conducted in the Department of Obstetrics and Gynecology at Azadi Teaching Hospital / Kirkuk during the period of 10 months from 1st of Feb till 1st of Dec 2019.Verbal permission was obtained from the patient.

The study included 90 pregnant women selected from outpatient clinic and those who were admitted to the labor room or emergency ward. Four participants showed invalid or missing anti-chlamydial antibody titer results, so the total number of participants included in the analysis was 86. They were divided into two groups:**Case group:** Included 43 women diagnosed with tubal ectopic pregnancy.**Control group:** Included 43 women with early normal intra uterine pregnancies (1st trimester) after matching for age and gestational age with case group. Diagnosis of ectopic pregnancy was done by the following ; Beta HCG level higher than 1500 IU/ML served as the diagnostic criteria for ectopic pregnancy,

Empty cervical canal and uterine cavity (but a gestational sac seen in either adnexia or tube by transvaginal ultrasound scan) ,

The ultrasound scans were carried out by an obstetrician while a radiologist verified the diagnosis, Some of patients diagnosed clinically in acute condition and confirmed by surgery and histopathology .Selection of normal pregnancies was done in women with menstrual delays, beta HCG and diagnosis confirmed by an ultrasound scan as normal intrauterine pregnancy.

Exclusion criteria: Women using an intrauterine contraceptive device (IUCD) at the time of conception, Previous history of ectopic pregnancy , history of infertility, history of tubal surgery, history of IVF, history of smoking, Other type of ectopic pregnancy, Heterotopic pregnancy.

A questionnaire had been applied to all study patients to collect needed information.General examination BMI was done for the patients.

The studied groups investigated for the following: Full blood count, Pregnancy test, Beta HCG titer, Serum anti chlamydial IgG antibody.

Examination by transvaginal ultrasound for site, size of the gestational sac and free fluid collection.

Significant unit (positive results) was recorded as > 11 NTU.

RESULTS

The total number of study participants was 86. They were divided into two groups: Case group included 43 women diagnosed as tubal ectopic pregnancy and control group included 43 women with early normal intra uterine pregnancies.

Table 1 shows the comparison between study groups by certain characteristics. In this study, no statistical significant differences ($P \geq 0.05$) in age, BMI, GA, and parity between study groups.

Table 1 : Comparison between study groups by certain characteristics

Variable	Study group		P – Value
	Case Mean ± SD	Control Mean ± SD	
Age (Year)	28.39 ± 5.8	27.72 ± 6.4	**0.609**
GA (Week)	6.46 ± 0.66	6.76 ± 1.3	**0.167**
BMI (kg/m²)	25.3 ± 2.6	25.67 ± 3.0	**0.545**
Parity	1.97 ± 1.5	1.69 ± 1.0	**0.313**

The comparison between study groups by anti-chlamydial antibody results is shown in figure (1) and table (2). In this study, 17 (39.5%) of patients who diagnosed with ectopic pregnancy showed positive results of anti-chlamydial antibody; while 7 (16.3%) of controls showed positive results of anti-chlamydial antibody.

We noticed that mean of anti-chlamydial antibody titer was significantly higher in patients who diagnosed with ectopic pregnancy than that in controls (11.72 versus 9.36 NTU, P= 0.001).

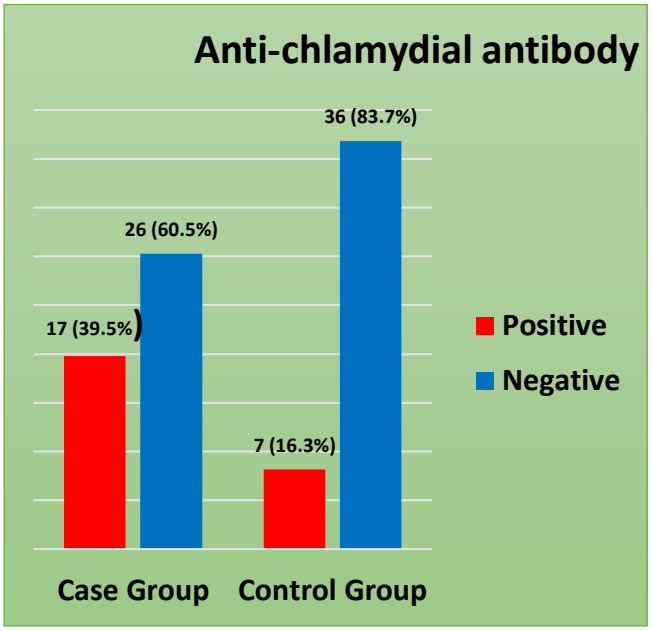

Figure 1 : Anti-chlamydial antibody results in study groups

Table 2 : Comparison between study groups by anti-chlamydial antibody titer

Anti-chlamydial antibody titer (NTU)	Study group		P – Value
	Case Mean ± SD	Control Mean ± SD	
	11.72 ± 1.81	9.36 ± 2.05	**0.001**

Receiver operating characteristic (ROC) curve analysis was constructed for anti-chlamydial antibody titer as a predictor for risk of ectopic pregnancy. As shown in figure (2) and table (3), the cut point of anti-chlamydial antibody titer was 9.98 NTU,

so anti-chlamydial antibody titer > 9.98 NTU may help to predict the risk ectopic pregnancy as a large significant area under the curve (AUC= 84.6%) indicating significant association between higher level of anti-chlamydial antibody titer and may help to perdict the risk of ectopic pregnancy.

Anti-chlamydial antibody titer > 9.98 NTU was 88.4% sensitive, 81.4% specific, and 84.9% accurate which may help to predict the risk of ectopic pregnancy.

Table 3 : Diagnostic accuracy for test of ectopic pregnancy

Anti-chlamydial antibody titer (NTU)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	9.98	88.4 %	81.4 %	82.6 %	87.5 %	84.9%

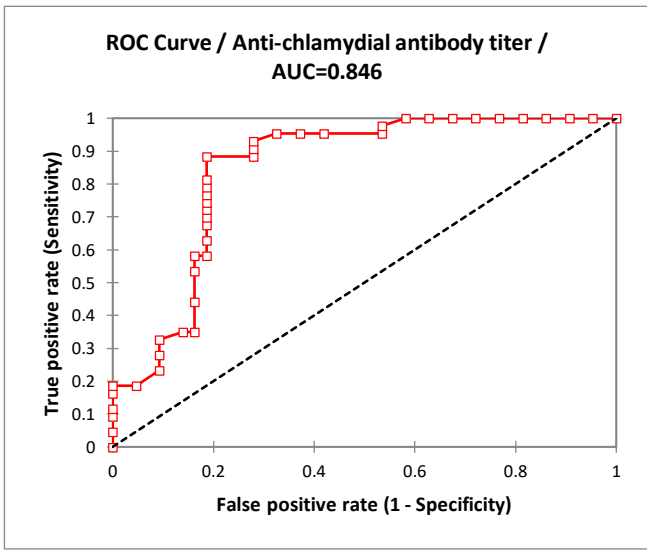

Figure 2 : ROC curve for anti-chlamydial antibody titer as a predictor for risk of ectopic pregnancy

No statistical significant correlations ($P \geq 0.05$) between anti-chlamydial antibody titer and all of age, BMI, and GA as shown in table (3.6).

Table 4 : Correlation between anti-chlamydial antibody titer and certain characteristics

Variable	Anti-chlamydial antibody titer (NTU)	
	r	P – Value
Age (year)	0.035	0.75
BMI (kg/m^2)	0.009	0.938
GA (Week)	- 0.059	0.592

DISCUSSION

Ectopic pregnancy remains a major public health problem especially in many developing countries where it is a significant contributor to pregnancy related morbidity and mortality [14]. This view is sustained by its rising incidence globally [15]. The main risk factors for tubal EP are damage to the tubes from surgery or injury, smoking and in vitro fertilization. Another important postulate for underlying cause of ectopic tubal may be the idea of change in transport media and environment of fallopian tubes leading to retention of the fetus [16].

A number of studies have looked at the antibody responses induced by C. trachomatis infection and have generally found a good correlation between serum antibodies to C. trachomatis and EP. In addition, EP risk was observed to be greatest amongst a subset of women with multiple exposures to chlamydial infections. These reports conclude that C. trachomatis infection is a major cause of fallopian tube damage which predisposes to EP [17].

The level of C. trachomatis IgG antibodies decreases with time since infection [17], but antibodies can persist at a detectable level for several years even after proper antibiotic treatment. Antibodies have noticed to be detectable longer in woman who have had repeated C. trachomatis infection, and a relationship between antibody titers and the severity of inflammation and tubal damage has been demonstrated [18].

In the current study, 86 patients enrolled. They were divided into two groups: Case group included 43 women diagnosed as tubal EP and control group included 43 women with early normal intra uterine pregnancies.

In our study, 39.5% of patients with EP showed positive results of anti-chlamydial antibody; while only 16.3% of controls had. The mean of anti-chlamydial antibody titer was significantly higher in patients who diagnosed with EP than that in controls (11.72 versus 9.36 NTU, P= 0.001). Additionally, in both case and control groups, no statistical significant association ($P \geq 0.05$) between anti-chlamydial antibody results and parity. And no statistical significant correlations between anti-chlamydial antibody titer and all of age, BMI, and GA ($P \geq 0.05$).

In comparison to other studies, there were agreement with study in Rantsi *et al* [19] study in 2016 Cases with EP were more likely than controls to be C. Trachomatis IgG seropositive in the index pregnancy samples (29.3 % vs. 15.0 %, $P < 0.001$. Agholor *et al* [20] study in 2013, in which reported that EP patients had a higher prevalence of exposure to Chlamydia trachomatis (48.0%) as evidenced by the presence of antibodies to Chlamydia trachomatis than did pregnant controls (16.3%). The difference was statistically significant ($p<0.001$), also they found that among the ectopic pregnant patients (compared with controls),

anti-chlamydia antibodies were more likely to be present in parous women relative to nulliparous, although associations were not statistically significant (P>0.05).

In comparison to the present study, Bokhari *et al* [21] in their study in 2017, in which a total number of 88 patients, comprising 44 cases (EP) and 44 controls (1st trimester normal intra uterine pregnancies) were included. In their results, regarding of anti-chlamydial antibody (IgG) distribution among cases and controls, of anti-chlamydial antibody was higher in case than control group, as detected in 25% of cases and in 11.3% of controls. The difference was statistically non-significant between them in regard to of anti-chlamydial antibody (p value = 0.097). On other hand, association between parity and anti-Chlamydia IgG among cases and controls was conducted and showed no significant association between both of them, the same is observed to the method of contraceptive (P > 0.05).

Differences observed among above studies can attributed to the sample size included in each study, added to that the virulence and duration of the previous disease, titer of antibody levels in participants with EP since it found that for unknown reasons that are not precisely known, seropositivity reduces after months and may fade out in some people [22], previous PID or other pelvic surgery and any evidence of adhesion from other surgeries, all of these can determined the changes observed above.

In the current study, ROC curve analysis, in which the cut point of anti-chlamydial antibody titer was 9.98 NTU, titer > 9.98 NTU may help to predict the risk of EP. Anti-chlamydial antibody titer was 88.4% sensitive, 81.4% specific, and84.9% accurate for prediction of EP. The exact mechanisms of C. trachomatis–induced reproductive health problems are unknown, but it has been shown that the sequelae associated with Chlamydia infection are largely due to the inflammation resulting in scarring and damage of infected mucosal tissue [23].

Both innate and adaptive immune responses have a role in the pathogenesis of chlamydial disease, and genetic variation on host immune response is suggested to play a role in determining why some women develop sequelae and others do not [24]. In some women, a single chlamydial insult may be sufficient to induce tubal damage, whereas most infected women seem not to develop clinical complications after a single episode of chlamydia salpingitis. However, persistent or multiple exposure to C. trachomatis can eventually produce irreversible tubal scarring [25]. Different Chlamydia serovars can also associate the intensity of symptoms, immune response, reinfection, or infection duration, which all may contribute to the pathology development [26].

No statistical significant differences in age, BMI, GA, and parity were found between study groups ($P \geq 0.05$). Abdullateef *et al* [27] found in their study in 2013 that mean and SD of age of women presented with tubal EP was 28.97 ± 0.957 years, also found that mean and SD of BMI was 26. 27 ± 0.735 Kg/m^2 in EP group while mean and SD of gestational age was 6.425 ± 0.142 weeks, and there were no significant differences between women with tubal EP and women with normal pregnancy in regard to gestational age. Furthermore, Al-Bayati *et al* [28] found that mean of age in women with EP group was slightly higher than in women with normal pregnancy (29.30±5.71) and (25.90±5.10 years) respectively, but no statistically significant difference among them (P=0.197).

Conclusion

1. This study showed that higher proportion of women with tubal EP had positive anti-chlamydial antibody than those with normal pregnancy.
2. Anti-chlamydial antibody titer may help to predict the risk of EP as the mean of anti-chlamydial antibody titer was significantly higher in patients who diagnosed with EP than that in women with normal pregnancy regardless age, gestational age or parity, especially in those patients who presented with chronic abdominal pain or vaginal spotting .

There is no conflict of interest between the authors

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Recommendation

1. Planning a future larger clinical trials is recommended with large number of sample and extended period to determine the following:

   -Ensuring how useful anti-Chlamydia antibody to predict patients at risk for ectopic pregnancy.

   - Determine whether combination of PCR with ELISA are more helpful to confirm the infection and guide further management.

2. Perform routine screening for Chlamydia trachomatis for those (sexually active women under 25 years old , sexually active women aged 25 years and older if at increased risk) to avoid future sequels.

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